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Food commensals as a potential major avenue in transmitting antibiotic resistance genes

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Abstract

The rapid emergence of antibiotic resistant (ART) pathogens is a major threat to public health. While the surfacing of ART foodborne pathogens is alarming, the magnitude of the antibiotic resistance (AR) gene pool in foodborne commensals is yet to be revealed. Incidence of ART commensals in dairy, meat, seafood and produce products was examined in this study. Twelve of the 15 retail cheese samples tested were found to contain Tet^r microbes ranging from 10^2 to 10^7 CFU per gram of food. All 15 retail produce samples examined contained Em^r microbes up to 10^7 CFU per gram of product. The presence of large populations of ART bacteria in these foods, particularly in many ready-to-eat, "healthy" food items, indicates that the ART bacteria are abundant in the food chain. AR-encoding genes were detected in ART isolates and the main hosts for these genes in several types of foods were further identified. Particularly, *Streptococcus thermophilus*, a commonly used dairy starter culture, was found to be a major carrier organism for AR genes in cheeses, arguing that it might no longer be suitable for cheese production. AR-encoding plasmids were isolated from several foodborne commensals and transmitted to the oral residential bacterium *Streptococcus mutans* via natural gene transformation under laboratory conditions, suggesting the possible transfer of AR genes from food commensals to human residential bacteria via horizontal gene transfer. Since the ART bacteria from food have the potential to modify the human ecosystems by becoming part of the microbiota, or involved in gene swapping during their transient passage through host oral, upper respiratory and GI systems, routine intake of large numbers of ART bacteria through food consumption may play a significant role in the existence of an increasingly antibiotic-resistant microbiota in the human ecosystems.

Introduction

The rapid emergence of resistant pathogens to various antibiotics indicates that the surfacing of resistant pathogens untreatable by antibiotics constitutes a real threat to public health (Levy 1998). To effectively combat this problem, establishing a comprehensive understanding of the major pathways in antibiotic resistance (AR) gene dissemination as well as the key mechanisms in the evolution of antibiotic resistant (ART) bacteria is an urgent need.

While horizontal gene transfer among pathogens in the hospital environment has been recognized as an important avenue for the rapid spread of AR genes among pathogens, evidence showed that additional pathways besides the clinical settings also contributed to the spread of ART bacteria, and that the use of antibiotics in animals can also impact human microbiota (Levy et al, 1976; Smith et al., 2002). Various reports on the isolation of ART pathogens from food animals, retail meat products and farm environment as well as the identification of AR genes in these isolates further support the notion that inappropriate use of antibiotics in agriculture and animal production, whether for therapeutic or subtherapeutic purposes, facilitated the emergence of ART pathogens and that the food chain could be another route in transmitting ART pathogens to humans. However, most food related studies, to date, have examined the AR profiles of a specific group of pathogens, such as *E. coli* O157:H7 (Schmidt et al., 1998; Kim et al., 1994;), *Campylobacter sp.* (Gaudreau and Gilbert, 1998; Ruiz et al., 1998; Smith et al., 1999, Ge et al., 2003), *Salmonella* enterica serovar Typhimuroim (Abrahim et al., 1998; Lee et al., 1994; Fey et al., 2000; Threlfall et al., 2000; Low et al., 1997; Chen et al., 2004), and *Listeria monocytogenes* (Charpentier et al. 1999; Poyart-Salmeron et al., 1992; Hadorn et al., 1993; Roberts et al., 1996; Biavasco et al., 1996; Abrahim et al., 1998). The studies on commensal bacteria were limited and primarily focused on the opportunistic pathogen enterococci (Klein et al., 1998; Coccoincelli

et al., 2003; Johnston and Jaykus, 2004). In almost all of these cases, a standard laboratory enrichment procedure (http://www.fda.gov/cvm/Documents/AppendicesA-6.pdf) is required in order to detect the presence of the ART bacteria. The difficulty in isolating ART bacteria in such investigations masks the real magnitude of the AR problem associated with the food chain.

The objective of this study is to investigate the distribution spectrum and magnitude of antibiotic ART commensal bacteria and AR gene pool in the US food chain, and to discuss its potential impact on public health. Unlike most previous investigations, this study targeted total food microbiota instead of a particular group of microorganisms or pathogens. Food samples were analyzed without any laboratory enrichment procedures. The goal was to detect bacteria resistant to two commonly used antibiotics, tetracycline (Tet) and erythromycin (Em), within the microflora associated with foods. These two antibiotics are heavily used in animal production, and are still therapeutic options for man (Chopra and Roberts 2001; Roberts 2004). The presence of several AR markers including *ermB*, *ermC*, *tet*S/M and *tet*A was examined in selected food isolates. The *tet*A gene encodes for the drug efflux protein TetA. The *tet*S/M, *erm*B and *erm*C genes encode proteins that can abolish the function of corresponding antibiotics by non-covalent modification (*tet*S/M) or methylation (*erm*B and *erm*C) of the ribosomes. These genes have previously been identified in both Gram-positive and Gram-negative microbes, wherein they are associated with various mobile elements (Roberts, 1998). Despite the fact that this current study only screened for a limited number of resistance markers, it certainly revealed the high prevalence of ART commensals and AR genes in various food items. These findings have direct implications on public health, as humans consume these commensal bacteria loaded foods on a daily basis.

Materials and Methods

Food sample preparation and enumeration of total and ART populations. Food samples were purchased from local grocery stores. All food items analyzed were within the expiration date for consumption. Five grams of each sample were aseptically removed from the product packaging and placed in disposable Ziploc bags containing 10 ml of sterile 0.1% peptone water. Bagged samples were then hand-massaged for 10 minutes. Homogenized samples or rinsing liquids were serially diluted and plated on non-selective Plate Count Agar (PCA, Becton Dickinson and Company, Sparks, MD) for non-selective total microbial counting, and on PCA plates containing 16 μg mL^{-1} of Tet or 8 μg mL^{-1} of Em (Fisher Biotech, Fair Lawn, NJ) for assessing Tet and Em resistant population. Serially diluted samples were also plated on Difco Lactobacilli MRS Agar (MRS, Becton Dickinson and Company) plates with the proper antibiotics to recover ART lactic acid bacteria, and on Pseudomonas Selective Agar (PSA, EMD Chemicals Inc., Gibbstown, NJ) with the proper antibiotics for ART *Pseudomonas* species isolation. Plates were incubated at 32°C or 20°C for up to 48 h at temperatures as specified for each sample. The cell numbers reported were the mean values from duplicates.

Detection of AR genes by conventional PCR. Conventional PCR was conducted to detect the presence of AR genes in the ART isolates. Bacterial cells from single colonies were re-suspended in 300 μl sterile dH_2O containing 100 μg of 1:1 mixture of 0.5 μm diameter and 0.1 μm diameter glass beads (Biospec Products, Inc, Bartlesville, OK). The sample mixtures were homogenized using the Mini-Bead-Beater-8 (Biospec Products, Inc, Bartlesville, OK) for 2

min at maximum speed. The resulting cell extracts were placed in a boiling water bath for 10-15 min and 5 µl of the supernatant was used as PCR templates. The PCR primers *tet*A-FP 5'GCTACATCCTGCTTGCCTTC3' and *tet*A-RP 5'CATAGATCGCCGTGAAGAGG3' were used to amplify the 220 bp *tet*A fragment (Ng et al., 2001), *tet*S-FP 5'CATAGACAAGCCGTTGACC3' and *tet*S-RP 5'ATGTTTTTGGAACGCCAGAG3' were for the 667 bp *tet*S/M fragment (Ng et al., 2001), *erm*B-FP 5'GGAACAGGTAAAGGGC3' and *erm*B-RP 5'GGTTTAGGATGAAAGC3' for the 389 bp *erm*B fragment (this study), and *erm*C FP 5'GCTAATATTGTTTAAATCGTCAAT3' and *erm*C RP 5'TCAAAACATAATATAGATAAA3' for the 640 bp *erm*C fragment (Chung et al., 1999). PCR was conducted using reagents as described previously (Luo et al., 2004) and the amplification conditions included an initial step of 3 minutes at 95°C and 35 cycles of 30 seconds at 95°C (melting), 30 seconds at 55°C (annealing), and 30 seconds at 68°C (extension), using a thermal cycler (iCycler™, Bio-Rad, Hercules, CA). PCR products with expected sizes were purified using a commercial purification kit (QIAquick®, Qiagen, Valencia, CA) following manufacturer's instruction. DNA sequences of the fragments were determined using a DNA analyzer (ABI PRISM® 3700, Applied Biosystems, Foster City, CA) at the Plant Genome Sequence Facility, The Ohio State University. The DNA sequences were compared with published Tet or Em resistance gene sequences deposited in the NCBI database.

Identification of ART isolates. ART isolates containing the resistance genes were identified by PCR amplification of the 16S rRNA gene fragment and sequence analysis following procedures as described previously (Connor et al., 2005). The 1.5 kb 16S rRNA gene fragment of the isolates were amplified using the primer pair 5'AGAGTTTGATCCTGGCTCAG

3' and 5'TACCTTGTTACGACTT 3' by PCR (Weisburg et al., 1991), and the sequences of the fragments were determined and compared with those deposited in the NCBI database.

MIC profiles of ART isolates. The minimum inhibition concentration (MIC) profiles of selected ART isolates were determined using the commercial kit Sensititre ® 18-24 Hour MIC and Breakpoint Susceptibility Plates (TREK Diagnostic Systems, Cleveland, OH) following the manufacturer's instructions, with modifications. MRS or brain heart infusion (BHI) broth instead of the standard Mueller-Hinton broth was used to culture fastidious organisms. The MIC panels were incubated at either 30^0C or 37^0C for 24-48 h. The MIC's were reported as the minimum concentration of the antibiotic that inhibited visible growth, as indicated by increased turbidity or by deposition of cells at the bottom of the wells. Control strains used in the study include *S. aureus* ATCC 29213 [American Type Culture Collection (ATCC), Manassas, VA], *P. aeruginosa* ATCC27853 (ATCC), *L. lactis* ML3 (Kuhl et al., 1979), and *S. thermophilus* LMD-9 (http://www.ncbi.nlm.nih.gov/entrez/query.fegi?db genomeprj&cmd Retrieve&dopt=Overview &list_uids=13773).

Plasmid isolation from selected antibiotic resistant isolates. In this study, the lactococcal strains CZ-T4 (Tet^r) and CZ-T8 (Tet^r) were isolated from commercial cheddar cheese, while strain RMK-T14 (Tet^r) was obtained from raw milk. The multi-drug resistant *L. lactis* K214 was isolated from cheese (Perreten et al., 1997). The strains were grown in either MRS broth or M17 broth with 0.5% glucose, supplemented with 5 μg ml^{-1} Tet and incubated at 30°C for 24 h. Plasmids were isolated from these strains following the method of Anderson and McKay (1983) and were used in the natural transformation experiments.

Natural gene transformation. Overnight cultures of *S. mutans* UA 159 grown in either BHI or Bacto Todd-Hewitt broth (Becton Dickinson and Company) were transferred to fresh medium and incubated at 37°C until the OD_{600} reading reached approximately 0.15-0.30. Transforming plasmid DNA was then added at a final concentration of 1 μg mL^{-1}. The cultures were allowed to grow for an additional 2 h at 37°C. After the incubation period, the cultures were briefly vortexed and plated on selective and non-selective BHI plates. For the selection of Tet^r transformants, BHI plates were supplemented with 5 μg mL^{-1} Tet. Plates were incubated in a 5% CO_2 incubator at 37°C for 48 h. Transformation efficiency was calculated based on the ratio of Tet^r transformants to the total number of viable cells.

Results

Prevalence of ART bacteria in food samples. Using the screening conditions as indicated, ART bacteria were detected in the majority of the retail food items examined (Table 1), except processed cheese and yogurt (data not shown). High counts of ART microbes were detected not only from raw food materials such as meat and shrimp, but also from many ready-to-eat food items. Twelve out of the 15 cheese samples analyzed contained Tet^r microbes ranging from 10^2 to 10^7 CFU g^{-1} of food. The number of Tet^r microbes was greater in cheeses than was Em^r bacteria. Among 15 produce samples examined (7 reported in Table 1), all contained up to 10^7 CFU ART microbes per gram of food, and majority of which (20 to 92%) were resistant to Em. The number of Tet^r microbes in produce was much lower than that of Em^r. Since it is unlikely that Em is used in produce production or Tet in cheese fermentation, the reasons for the difference in the magnitudes of Tet and Em-resistant microbes in these ecosystems are unknown.

Studies are currently undergoing in our laboratory to reveal the possible factors contributing to the selective maintenance of these ART bacteria in the corresponding ecological niches.

It is worth of noting that the study was conducted using limited incubation conditions, the antibiotic concentrations used to screen for resistant organisms were based on those used for enterococci and might not be optimal for all bacteria, and bacteria located within the plant tissues may not have been detected. Therefore the numbers reported here only represent a portion of the total ART bacterial load in these foods.

Detection of AR genes and ART isolates identification. To confirm that most of the ART organisms detected by growth on the selective agar plates were resistant bacteria due to the possession of various resistance mechanisms, conventional PCR was conducted to detect the presence of selected AR genes in these organisms. Table 2 summarizes the screening results for some of the Em and Tet markers, and the identities of selected ART isolates as determined by 16S (bacteria) rRNA gene sequence analysis.

Among the Tet^r isolates recovered from cheese, more than 20% contained the *tet*S/M gene (Table 2). The 16S sequence analysis showed that isolates CZ-T4 and CZ-T8 had 97% sequence identity to unidentified *Lactococcus sp.*, and particularly had 93-94% identity to *L. garvieae* and *L. lactis*. Meanwhile, the 16S rRNA sequence analysis of the raw milk ART isolate RMK-T14 showed similar sequence identity to unidentified *Lactococcus sp.* and *L. garvieae*, suggesting this is a common organism from milk. Therefore, it is possible that the cheese lactococcal *tet*S/M-containing resistant isolates were originated from milk (pasteurized but not

sterile) or dairy processing environment during cheese fermentation. *S. thermophilus* was found to be an important carrier for the *tet*S/M gene in cheeses.

Among the Em^r isolates from cheese, more than 50% contained the *erm*B gene, and the carrier organisms identified so far include *Staphylococcus* sp. (5 out of 28) and *S. thermophilus* (23 out of 28). Approximately 10-30% of the Em^r isolates from salad and carrots contained the *erm*B gene, with the primary carrier organisms being *Pseudomonas* sp. or *Enterobacter* sp.

In addition, the *tet*A gene was identified from two cheese isolates CZ-T3, CZ-T7 and several isolates from raw pork meat. These isolates were all identified as *Pseudomonas* sp. Both *tet*S/M and *erm*C genes were found in the isolate CX-1 EM from packaged sliced chicken lunchmeat, suggesting a multi-drug resistance phenotype of the strain. CX-1 EM was identified to be *Pseudomonas* sp. ART bacteria were isolated sporadically in lunchmeat (data not shown), which is probably due to occasional contamination during the processing of the meat.

MIC analysis. MIC tests of selected cheese isolates showed that *Lactococcus sp.* CZ-T4 and CZ-T8 (*tet*S/M^+) were resistant to at least 16 μg mL^{-1} Tet, and *S. thermophilus* T4 (*erm*B^+) was resistant to Em (≥ 8 μg mL^{-1}), clarithromycin (≥ 8 μg mL^{-1}), and clindamycin (4 μg mL^{-1}). *Staphylococcus sp.* C202 was resistant to both Em (≥8 μg mL^{-1}) and Tet (≥16 μg mL^{-1}), suggesting the possible possession of both resistance determinants in this isolate.

Lactococcus sp. RMK-T14 (*tet*S/M^+) from raw milk was resistant to Tet (≥16 μg mL^{-1}), Em (≥8 μg mL^{-1}), clarithromycin (≥8 μg mL^{-1}), and clindamycin (≥4 μg mL^{-1}). Therefore this

isolate likely carried multi-drug resistant determinants or multi-drug resistant mechanism(s). The raw milk isolate *Streptococcus uberis* RMK-T22W exhibited resistance to Tet (≥16 μg mL^{-1}).

All of the *Pseudomonas tetA*$^{+}$ isolates recovered from pork and cheese exhibited resistance to Tet (≥16 μg mL^{-1}), Em (≥2-8 μg mL^{-1}), and vancomycin (≥32 μg mL^{-1}), indicating a multi-drug resistance phenotype in these organisms. The *Pseudomonas sp.* CX-I EM (*ermB*$^{+}$*tet*S/M^{+}) from packaged sliced chicken lunchmeat was resistant to Tet (≥16 μg mL^{-1}) and Em (≥8 μg mL^{-1}).

Horizontal transfer of the AR gene from food isolates to oral residential bacterium. The *tetS*-containing lactococcal isolates CZ-T4 and CZ-T8, recovered from cheese, and RMK-T14, isolated from raw milk, contained a plasmid with an approximate size of 20-25 kb. To assess the potential risk of the foodborne ART bacteria in disseminating AR genes to human microbiota, plasmids isolated from the above strains were used for natural transformation of the oral cariogenic pathogen *S. mutans* in laboratory media. The *tet*S/M gene was successfully transferred to *S. mutans* UA159 at frequencies ranging from 1.9 x10^{-7} to 2.8 x 10^{-5}, 4.7 x10^{-7} to 2.3 x 10^{-6}, and 3.8 x 10^{-7} to 2.1 x 10^{-6} transformants per recipient cell using CZ-T4, CZ-T8 and RMK-T14 plasmid extracts, respectively. In addition, the multi-drug resistant plasmid pK214 from the cheese isolate *L. lactis* K214 was also successfully transformed into *S. mutans* UA159 at frequencies of 1.1 x 10^{-6} to 1.2 x 10^{-5} transformants per recipient cell. PCR amplification confirmed the presence of the *tet*S/M gene in the streptococcal transformants. MIC test showed that the transformants had significantly increased resistance to Tet (≥16 μg mL^{-1}) compared to the parental strain UA159 (2 μg mL^{-1}). These results illustrated that the *tet*S/M gene from food

isolates can lead to resistance in residential host bacteria or pathogens, if acquired by horizontal gene transfer.

Discussion and Conclusion

The increasing inability to effectively treat many infections with antibiotics due to a rapidly emerging AR phenotype in many bacteria is a major threat to public health. Effective control strategies to reduce antibiotic resistance need to be built upon having a complete understanding of the key pathways leading to evolution and spread of AR genes as well as identifying the carrier organisms. It has been known that the presence of AR gene pool is the basis for horizontal gene transfer and that selective pressure (antibiotic usage) plays an important role in the enrichment of ART bacteria via horizontal gene transfer (Levy 1998). Horizontal gene transfer among pathogens in the hospital environment is recognized as a main vehicle for the rapid spread of AR genes among pathogens, whereas minimizing antibiotic misusage has been the primary control strategy used to combat the AR problem worldwide. The potential impact of antibiotics used in animal production on the emergence of ART pathogens has also been discussed extensively. However, recent studies have showed that the microbiota in children and adults is becoming increasingly resistant to antibiotics, even in the absence of antibiotic treatment (Lancaster et al., 2003, 2005; Ready et al., 2003; Villedieu et al., 2004). Reports on AR gene reservoirs in the environment (Gilliver et al., 1999; Österblad et al., 2001; Nandi et al., 2001; Smith et al., 2004), combined with the important new results presented in this study, further indicate that the scope for AR gene transmission is quite broad.

This study targeted the AR gene reservoir in commensal bacteria associated with the food chain. The size of the AR gene pool is quite large in commensals, and horizontal transmissions in ecosystems, directly or indirectly mediated by the abundant and diverse commensal populations, are much more likely events than direct AR gene dissemination from one pathogen to another. Commensal bacteria could even serve as "enhancer" facilitating the dissemination of AR genes in ecosystems (Luo et al, 2005). Our data on the prevalence of ART bacteria can be translated to 10^3 to 10^8 CFU Tet^r microbes per slice of cheese (about 20 g), and up to 10^9 CFU g^{-1} Em^r microbes per serving of salad (about 50-100 g) or baby carrots (about 10 pieces). Since these foods are normally considered healthy, and are consumed without further cooking or processing, these data are a good indication of the daily intake of ART bacteria via the food chain. Therefore without even being exposed to the hospital environment, human beings are unintentionally and constantly inoculated through intake of food with large populations of ART bacteria including opportunistic pathogens and commensals such as *Pseudomonas* sp., *Streptococcus* sp. and *Staphylococcus* sp., many of which carry resistance determinants to antibiotics. This finding is consistent with a previous report that consuming sterile foods can significantly decrease the presence of ART bacteria in the GI system (Levy 1998). Particularly, oral cavity could be an important area where many initial interactions between food microbes and human microbiota, including horizontal gene transfer events such as conjugation and transformation, took place during the retention of food residues in the oral cavity. In fact, the *tet*S/M and *erm*B genes were found to be abundant in bacteria isolated from foods, which is in agreement with the prevalence of these Tet- and Em-resistance genes in human oral microflora (Roberts, 1998). Successful transmission of the resistance genes from the food isolates to the oral residential bacterium *S. mutans*, by natural gene transformation, further confirmed the functionality of the mobile

resistance-encoding elements, if acquired by horizontal gene transfer. Further research is needed to establish the direct correlation between the ART microbes from foods and the ART population in the host ecosystems. However, it is evident that a constant supply of ART bacteria, partnered with occasional colonization, and horizontal gene transfer are at least partially responsible for the increased AR profiles seen in human.

While the ART population in the human ecosystems might not cause a major problem in healthy people, such an intrinsic AR gene pool could have significant impact on pathogen resistance in susceptible population, and particularly those receiving antibiotic treatment. Due to the magnitude and spectrum of the ART bacteria identified in foods, we propose that in addition to the medical route, the food chain might have served as a major avenue for the transmission of ART bacteria from the environment to human in the general population.

It is worth noting that ART pathogens can emerge in the natural ecosystems and be transmitted to the host, or directly evolve within the host ecosystems. Furthermore, not only are food and related processing environment part of the external ecosystems where ART bacteria could evolve, but also foods are major carriers for ART bacteria and selective pressures inherent to the processing procedures themselves (such as addition of preservatives or other food additives), which could further augment the emergence of ART bacteria in the host ecosystems. While it is a major challenge to track the direct and indirect gene transfer events among microbes in complicated ecosystems (Andremont 2003), identifying key AR gene carrier organisms in foods not only reveals the ultimate consequence of these events in the food chain and the organisms involved in horizontal gene transfer, but enables further characterization of conditions

in these ecosystems that might facilitate horizontal gene transfer and features of the organisms that might grant their fitness in such ecological niches (Luo et al., 2005a). Such understanding would be critical for effective counteractive strategies to interfere with the detrimental gene swapping in both natural and host ecosystems.

Identification of the key pathways in AR gene transfer is critical but developing a strategy to combat this problem is even more important. Among the foods examined, meat and seafood products likely are subject to heat treatment and the ART number would be significantly reduced in cooked foods. Read-to-eat items such as salad and baby carrots are normally consumed raw. Therefore including a bactericidal procedure is important before consumption. Preliminary studies in our group showed that applying minimal heat for short periods (within seconds) or treatments with active ingredients can effectively destroy the ART flora in these products (Lehman et al, unpublished data). Such treatments will not change the sensory feature of the products and will likely lead to manageable approaches for the industry and the consumer to combat the AR problem. The finding of ART bacteria in cheeses often associated with raw milk, such as *Lactococcus sp.*, *Streptococcus sp.* and *Staphylococcus sp.*, suggests that cheese fermentation is a susceptible process during which ART bacteria could evolve and proliferate. Improving sanitation and milk heat treatment are thereby an essential step in reducing ART bacteria. Fortunately, traditional starter and adjunct cultures such as *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *Lactobacillus* sp. so far are not among the identified carrier organisms for AR genes. However, an industrially important lactic acid bacterium, *S. thermophilus*, was found a dominant carrier organism for both Tet and Em genes. Genetic screening and MIC tests of three commercial *S. thermophilus* starter cultures showed that the strains are free of these AR

genes, suggesting that the susceptibility of this organism to horizontal gene transfer during cheese fermentation and its unsuitability as cheese starter culture.

In general, while it would be a tedious and likely long-term effort to clean up the AR gene pool in the environment, interrupting the transmission of ART bacteria into human by focusing our efforts on the food chain could be an effective strategy to combat the AR challenge in human.

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Table 1. Prevalence of AR microbes in selected food samples

Food Item	Sample Source	Total Plate Count (CFU/g food)	Tet-resistant Count* (CFU/g food)	Em-resistant Count§ (CFU/g food)
Cheddar Cheese #1[a]	Store I (Brand A)	$3.2\times10^{7}\pm1.4\times10^{6}$	$1.1\times10^{3}\pm1.4\times10^{1}$	$9.0\times10^{1}\pm1.4\times10^{1}$
Cheddar Cheese #2[a]	Store II (Brand B)	$2.9\times10^{6}\pm9.1\times10^{5}$	$1.2\times10^{3}\pm6.7\times10^{2}$	$9.5\times10^{2}\pm4.2\times10^{1}$
Cheddar Cheese #3[a]	Store II (Brand C)	$2.5\times10^{8}\pm3.1\times10^{7}$	$4.1\times10^{7}\pm7.0\times10^{5}$	$4.3\times10^{3}\pm1.3\times10^{3}$
Cheddar Cheese #4[a]	Store II (Brand D)	$5.5\times10^{6}\pm3.5\times10^{6}$	$1.1\times10^{3}\pm1.0\times10^{1}$	0
Cheddar Cheese #5[a]	Store I (Brand E)	$1.9\times10^{8}\pm4.0\times10^{7}$	$8.0\times10^{3}\pm3.2\times10^{3}$	$4.0\times10^{1}\pm4.0\times10^{1}$
Cheddar Cheese #6[a]	Store I Brand (B)	$7.0\times10^{7}\pm2.8\times10^{7}$	$1.8\times10^{2}\pm2.0\times10^{1}$	$2.0\times10^{1}\pm2.0\times10^{1}$
Cheddar Cheese #7[a]	Store III Brand (B)	$3.9\times10^{7}\pm9.1\times10^{6}$	$3.7\times10^{4}\pm1.5\times10^{4}$	$3.7\times10^{2}\pm7.0\times10^{1}$
Cheddar Cheese #8[a]	Store I (Brand A)	$2.3\times10^{8}\pm3.6\times10^{6}$	$1.6\times10^{3}\pm2.0\times10^{2}$	<1
Cheddar Cheese #9[a]	Store III (Brand C)	$5.2\times10^{8}\pm5.2\times10^{6}$	<1	$1.0\times10^{1}\pm1.0\times10^{1}$
Colby Cheese #1[a]	Store I (Brand C)	$5.6\times10^{8}\pm4.4\times10^{7}$	$2.3\times10^{3}\pm6.1\times10^{2}$	$6.0\times10^{1}\pm0$
Colby Cheese#2[a]	Store II (Brand B)	$2.2\times10^{8}\pm8.5\times10^{6}$	$2.8\times10^{6}\pm8.6\times10^{5}$	$2.2\times10^{3}\pm1.4\times10^{2}$
Swiss Cheese #1[a]	Store I (Brand E)	$2.4\times10^{8}\pm1.1\times10^{7}$	$4.7\times10^{5}\pm4.4\times10^{4}$	$2.7\times10^{2}\pm1.0\times10^{1}$
Mozzarella Cheese #1	Store I (Brand B)	$3.6\times10^{6}\pm7.6\times10^{5}$	$5.4\times10^{3}\pm2.0\times10^{1}$	<1
Mozzarella Cheese #2	Store I (Brand A)	$1.3\times10^{8}\pm1.7\times10^{7}$	<1	<1
Mozzarella Cheese #3	Store I (Brand B)	$4.2\times10^{5}\pm4.9\times10^{4}$	<1	<1
Baby Carrots #1[c]	Store I (Brand F)	$1.2\times10^{6}\pm2.5\times10^{5}$	-	$6.7\times10^{5}\pm1.0\times10^{4}$
Baby Carrots #2[c]	Store II (Brand J)	$4.5\times10^{7}\pm1.4\times10^{6}$	$1.6\times10^{4}\pm7.1\times10^{2}$	$1.2\times10^{7}\pm1.4\times10^{5}$
Baby Carrots #3[c]	Store III (Brand K)	$9.0\times10^{7}\pm6.6\times10^{7}$	$4.1\times10^{5}\pm3.5\times10^{5}$	$4.0\times10^{7}\pm1.9\times10^{7}$
Mushroom #1[b]	Store I (Brand G)	$9.3\times10^{6}\pm1.4\times10^{5}$	$2.4\times10^{5}\pm4.2\times10^{3}$	$8.1\times10^{6}\pm1.4\times10^{5}$
Mushroom #1[c]	Store I (Brand G)	$1.5\times10^{5}\pm1.1\times10^{4}$	$5.0\times10^{1}\pm1.4\times10^{1}$	$1.0\times10^{5}\pm5.7\times10^{3}$
Salad #1[b]	Store II (Brand H)	$2.4\times10^{6}\pm8.5\times10^{4}$	$1.9\times10^{4}\pm7.1\times10^{2}$	$2.2\times10^{6}\pm1.4\times10^{5}$
Salad #1[c]	Store II (Brand H)	$6.5\times10^{5}\pm1.0\times10^{5}$	$2.8\times10^{3}\pm4.5\times10^{2}$	$5.4\times10^{5}\pm2.8\times10^{4}$
Salad #2[b]	Store I (Brand I)	$3.0\times10^{7}\pm2.8\times10^{5}$	$4.0\times10^{5}\pm1.7\times10^{4}$	$1.5\times10^{7}\pm5.7\times10^{5}$
Salad #2[c]	Store I (Brand I)	$1.7\times10^{6}\pm7.1\times10^{4}$	$6.0\times10^{2}\pm2.8\times10^{1}$	$1.4\times10^{6}\pm1.4\times10^{4}$
Salad #3[b]	Chain Restaurant A	$4.8\times10^{7}\pm5.7\times10^{5}$	$5.6\times10^{5}\pm2.8\times10^{5}$	$3.7\times10^{7}\pm2.3\times10^{6}$
Salad #3[c]	Chain Restaurant A	$2.0\times10^{7}\pm7.1\times10^{5}$	$4.8\times10^{3}\pm3.4\times10^{2}$	$5.2\times10^{6}\pm3.1\times10^{6}$
Shrimp #1[b]	Store III (Brand C)	$1.3\times10^{4}\pm1.4\times10^{2}$	$6.9\times10^{2}\pm1.4\times10^{1}$	$1.5\times10^{3}\pm2.8\times10^{2}$
Shrimp #1[c]	Store III (Brand C)	$9.3\times10^{2}\pm9.9\times10^{1}$	$3.4\times10^{2}\pm2.8\times10^{1}$	$2.1\times10^{3}\pm1.4\times10^{1}$
Shrimp #2[b]	Store IV	$4.3\times10^{3}\pm1.6\times10^{2}$	$1.2\times10^{2}\pm2.8\times10^{1}$	$3.1\times10^{2}\pm4.2\times10^{1}$
Shrimp #2[c]	Store IV	$1.3\times10^{4}\pm1.8\times10^{2}$	$6.0\times10^{1}\pm2.8\times10^{1}$	$1.4\times10^{2}\pm5.7\times10^{1}$
Pork Chop[b]	Store II	$3.2\times10^{4}\pm1.6\times10^{3}$	$5.7\times10^{3}\pm1.4\times10^{2}$	$1.8\times10^{4}\pm2.8\times10^{2}$
Pork Chop[c]	Store II	$4.6\times10^{2}\pm2.8\times10^{1}$	$1.0\times10^{1}\pm1.4\times10^{1}$	$6.0\times10^{1}\pm2.8\times10^{1}$
Raw Milk[d]	Pilot Plant	$4.5\times10^{3}\pm2.5\times10^{3}$	$3.4\times10^{2}\pm3.5\times10^{1}$	$3.4\times10^{2}\pm8.0\times10^{1}$
Raw Milk[a]	Pilot Plant	$8.5\times10^{3}\pm1.5\times10^{3}$	$4.8\times10^{3}\pm2.0\times10^{1}$	$7.6\times10^{3}\pm2.0\times10^{3}$
Raw Milk[b]	Pilot Plant	$7.6\times10^{2}\pm3.5\times10^{1}$	$7.0\times10^{1}\pm1.2\times10^{4}$	-

*Screened on agar plates containing 16μg/ml tetracycline.
§Screened on agar plates containing 8μg/ml erythromycin.
[a]Microorganisms were recovered from cheese samples by plating on MRS agar plate and incubated at 30°C.
[b]Microorganisms were recovered by plating on PCA agar plates and incubated at 20°C.
[c]Microorganisms were recovered by plating on PCA agar plates and incubated at 37°C.
[d]Microorganisms were recovered by plating on MRS agar plates and incubated at 20°C.

Table 2. Identification of antibiotic-resistant isolates from food based on 16S rRNA gene sequencing.

Food	ART trait	Resistance gene (# carriers /# isolates screened)	16S rRNA gene identity (#organisms/ #identified)
Cheese	Tet	*Tet*S/M (8/33)	*Lactococcus* sp. (2/8) *Streptococcus thermophilus* (5/8)
		*tet*A (2/33)	*Pseudomonas* sp. (2/2)
	Em	*erm*B (32/56)	*Staphylococcus* sp. (5/28) *Streptococcus thermophilus*. (23/28)
Raw milk	Tet	*tet*S/M (8/108)	*Lactococcus* sp. (1/8) *Streptococcus* sp. (1/8)
Salad	Em	*Erm*B (7/20)	*Enterobacter* sp. (3/4) *Pseudomonas* sp. (1/4)